1645

PATENT
Customer No. 22,852
Attorney Docket No. 03495-0203-00000

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

#12

In re Application of:

ROGNER et al.

Application No.: 09/847,665

Filed: May 3, 2001

For: IDENTIFICATION OF NEURAL DEFECTS ASSOCIATED WITH THE NUCLEOSOMAL ASSEMBLY PROTEIN 1I2

Group Art Unit: 1645

Examiner: Not yet assigned

Box PG-PUB

Commissioner for Patents
Washington, DC 20231

Sir:

REQUEST FOR CORRECTED PATENT APPLICATION PUBLICATION UNDER 37 C.F.R. § 1.221(b)

On August 1, 2002, the U.S. Patent and Trademark Office published this application as Publication Number US 2002/0102566 A1. The published application contains mistakes that are the fault of the Office and are, in Applicants' view, material. Attached hereto is a copy of the relevant pages of the originally filed application and a marked-up copy of the corresponding pages of the published application containing the mistakes.

A mistake is material when it affects the public's ability to appreciate the technical disclosure of the patent application publication or determine the scope of the provisional rights that Applicants may seek to enforce upon issuance of a patent. *See* 37 C.F.R. §

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1.221 (b). The mistakes listed below may affect the public's ability to appreciate the technical disclosure of the patent application publication or to determine the scope of the provisional rights.

The mistakes, which are indicated in red ink on the relevant pages of the marked-up copy of the published application attached hereto, are listed below with their corrections.

In Figure 5, portions of the panels depicting the invention are missing. Please refer to the copies of the Figures provided.

In Figure 7, [SEQ ID NO: 1] should be indicated after the sequence provided.

In Figure 8, [SEQ ID NO: 2] should be indicated after sequence provided.

In Figure 9, the first base, T, is cut off. In addition, [SEQ ID NO: 3] should be indicated after the sequence provided.

In Figure 10, the sequence should be labeled as "human" not "numan." In addition, [SEQ ID NO: 4] should be indicated after sequence provided.

On line 3 of paragraph [0004], the term "laevis" should written instead of "laevish."

On line 7 of paragraph [0133] the term "Nap1IL2/NAP1L2" instead of "Nap1L2/ NAP1L2."

On page 15, SEQ ID NO: 6 should be moved to the end of claim 45 on page 18. In addition, certain nucleotides in SEQ ID NO: 6 should be corrected, as follows:

Nucleotide 43 on line 10. should be a "c" instead of a "g";

Nucleotide 17 on line 18. should be a "g" instead of an "a";

Nucleotide 17 on line 25. should be a "G" instead of a "C";

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Nucleotide 44 on line 26. should be a "G" instead of a "C";

Nucleotide 50 on line 36. should be a "G" instead of a "C";

Nucleotide 76 on line 37. should be a "g" instead of a "c";

Each of these mistakes are clearly material as they impede the public's ability to appreciate the technical disclosure of the patent application publication. For at least this reason, the mistakes should be corrected.

Applicants request that the Office correct the above-identified mistakes in the published application, which are the fault of the Office. Further, Applicants request that the Office forward to Applicants a copy of the corrected published application or at least a notification of the occurrence or predicted occurrence of the corrected publication once it has been corrected.

Applicants believe that no Petition or fee is due in connection with this Request; however, if any Petition or fee is due, please grant the Petition and charge the fee to our Deposit Account No. 06-0916.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW,
GARRETT & DUNNER, L.L.P.

Dated: September 30, 2002

By: _____

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PAGES OF APPLICATION AS FILED

E17.5

E14.5

E12.5

a

b

c

E10.5

e

E9.5

FIG. 7

Sequence clone *Bpx* promoter murin SpeI-SalI fragment

ACTAGTCATATAGCTGGCTCTTTTACAAAAGGCTTCAACACCCCTCCCCC
CACACTTTAGTCATCCGTCATCTCTTCCTCATCAGGAAATATTATGAGAA
TTTTCCCATTTAAAATCACACAGGTTGTGAAAATTACAGAAACCAGGGTA
CAGAATATTTAAACCACTGTCAGTTACATCATCCAAAGGCCACCTATGCT
TATTTTTGGTAATTTTAAACCTCAAAGGATCTCTTTGTGGGCTCCTCCACT
ACCCTCCTCTCTTTCCCAGAGCCTCAGGTTATAACCAAAGGGATAGACTA
AAGACAATCCAGTACCTTGCCCATTTTTTTCATTCCTTGTCACTGTTTCCA
TATAGCTCTTTTGAAATTATGAACATATAGTATCAGTTGAAAACGGAATG
AATGATACTGCATTTCTGCAAAATTCCACAGGCTATAGGGTGGAAGATG
AGCCATAGGTGGAGGAATCAGCCATATTAGAGAATCTGGGAAGGCAAG
AGGTGTTGAAATTTTGATTCATCTACTAATTTACTGGCTCAGGATTTGTC
AATCACTGCAGCCTGGCAAATGAGATTAGAGAAGAGTCCTGGGAGGGA
AGGGGTGACGCAGCAACCTGCATACACTTAAAAAAAAAAGAGCTGAGAG
ACAACTGCGTAATCATACTGCGGCACCAGTTCCTCCATCCCTCCGCCCCC
GAGTGGCTGGAGCAGCTGCTTGCGGAGGTCTGCCCACTGCGGCTCTCTG
CAGTCTCTAGCCTGTTCCTTCAGGGCCTAGAGTCTCCGCCCAGACAGCCG
GTTTCAATTCTGCTATCCCAGCTTCAGCACCGTCTTTTATACTGCTTGCTG
CCTGCCATCAGTGCAGCCGCCGCCGCCTCTTGGTTCATCTCTGCCAGATC
ATCGCGCATCTGCTGTATTGGTGAGTCTTCCTGCGGAGGTCAGGTCTCCT
GATCTGCGGGCTTAGCCACCATAAGTGCAGGCGATCGTTTGAAAACAAT
GGCTGAATCAGTCGACCTCGAGGGGGGGCGTACCTTGCCCATTTTTTTCA
TTCCTTGTCACTGTTTCCATATAGCTCTTTTGAAATTATGAACATATAGTA
TCAGTTGAAAACGGAATGAATGATACTGCATTTCTGCAAAATTCCACAG
GCTATAGGGTGGAAGATGAGCCATAGGTGGAGGAATCAGCCATATTAGA
GAATCTGGGAAGGCAAGAGGTGTTGAAATTTTGATTCATCTACTAATTTA
CTGGCTCAGGATTTGTCAATCACTGCAGCCTGGCAAATGAGATTAGAGA
AGAGTCCTGGGAGGGAAGGGGTGACGCAGCAACCTGCATACACTTAAA
AAAAAAGAGCTGAGAGACAACTGCGTAATCATACTGCGGCACCAGTTCC
TCCATCCCTCCGCCCCCGAGTGGCTGGAGCAGCTGCTTGCGGAGGTCTG
CCCACTGCGGCTCTCTGCAGTCTCTAGCCTGTTCCTTCAGGGCCTAGAGT
CTCCGCCCAGACAGCCGGTTTCAATTCTGCTATCCCAGCTTCAGCACCGT
CTTTTATCCCCACTGCTTGCTGCCTGCCATCAGTGCAGCCGCCGCCGCCT
CTTGGTTCATCTCTGCCAGATCATCGCGCATCTGCTGTATTGGTGAGTCT
TCCTGCGGAGGTCAGGTCTCCTGATCTGCGGGCTTAGCCACCATAAGTG
CAGGCGATCGTTTGAAAACAATGGCTGAATCAGTCGAC

[SEQ ID NO:1]

FIG. 8 cont.

CGTACTTACATAATTCCAAGATCAGTGTTATTTTTCTCAGGAGATGCACT
TGAATCTCAGCAGGAGGGTGTAGTTAGGGAAGTTAATGACGAAATATAT
GACAAAATTATTTATGATGATTGGATGGCTGCAATTGAAGAGGTTAAAG
CCTGTTGCAAAAATCTTGAGGCATTAGTAGAAGATATTGATCGTTAAAAC
AGAGTAGATGCTTTTGAAACTAACTGCTCTACATGCAGTTACTGAAGACA
TAAGCAGTTAATATTGTCTTGTGTTCTGCATTTTTTCCTGTCATGCCAGTT
TAAAAATTCAAATACTAATTAATCTGACCTTGCATTGTAGTGGTATGATG
TTTTCAAGACATGTAGACTGTGATAAATGATTAAGACATTAATAGTCTGT
AGTATAACCCTTCTGAAGTCCTTGTGCCATGTATCTATTAATCTGTGGCT
GTGAATATTATTAGAAGTGCTAAATGAGATTATTTGTTTGCAAAGAAAAT
ATTGGAAACCTACCTAAGAGTGCTTTGCTATTTTCCCCCTTATCCTCTTAG
TGCTTTGGCCAATTGACTTTATTGTGCCTGCTTCATTTTGCAGTAAATATG
CAGTAGAATTTAAAACTTGAATGCCTAAGAGGCCTGCATATGATTGAGA
ATTTCAGGCAAAATCATATTTATTATTGATAACAGCTAGTGCAAGGCTTC
TGATTGTATGTGACTGTGATAAATAATAAAACTCAATTGTATTGAAGTTA
CTGTTTATCATTGACATGTGAGTTACAGTATTTTCAAATGTTGCAAATATT
GTCCTGTGTAATTGTGTAAACTGTGATTACAGTGTACATTTTTTTCATAAT
ATACTGAATCATTCATTGAAATGGACACTTTACCATTTCTGAAAATACAT
TTCATATTCTGTTCATTCACTGAAAAATAAAATGAATAAAAATTT

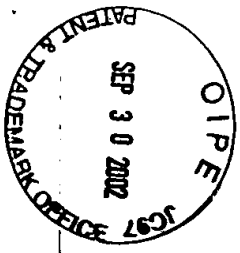
[SEQ ID NO:2]

FIG. 9

***BPX* human cDNA identical to genomic DNA**

TGTTAGAGAGCCTGGGAAGGTGAGcAGAGcTGAAAACTTGATAGATCTA
ATAATTTACTGGCTCTGGGTTTGTCAGTCACTACATTGCAGCAAATGAGA
TTAGAGCATAGTTGTGGGAGGGAAGGAGGTGACGCAGCAATCTATTTGC
ACCTAGAAATTTTAGGCAAGTGATAGCTGCGTAATCATACTGCGGCACC
GTTTTTTTCTTGCAGCAGTAGCTGCTTGCGGAGGAGGTCTGCCCACTGCA
GCTCTCTGCAGTCTCCGGCTCTCTCCTGCAGGATCGGTCAACGCAGCCGT
CGCCGCCCTCTGCACCCAGCCCAGGTCGCCACTGCTTCAGTCCGGTTCTC
AAAGCCTCAGCACCATCTTTTATCCCCGAGCAGCCTGGATCGTCGTTCCC
TCAGTCCGGACGCCACTGCTAGGTCCGACCACCGCCGCTTCTGATATTTC
GGTGAGTCTTTTCCTGTGGAGGTTTGGTCTCCCGATCTCTGTGGTAGCCA
CCTTAGGCGTGTACGGTCCTTTGAAAAATGGCCGAGTCAGAGAACCGCA
AGGAGCTGTCAGAATCCAGTCAAGAAGAGGCTGGTAATCAGATAATGGT
GGAAGGGCTCGGGGAACATCTGGAGCGCGGTGAAGATGCCGCTGCTGG
GCTTGGAGACGATGGGAAGTGCGGTGAAGAAGCTGCCGCTGGGCTTGG
GGAAGAAGGGGAAAACGGTGAAGATACTGCTGCTGGGTCCGGGGAAGA
TGGGAAAAAAGGTGGCGATACTGATGAGGACTCAGAGGCAGACCGTCC
AAAAGGACTTATC
GGTTATGTTTTAGATACAGACTTTGTTGAAAGTCTACCTGTGAAAGTTAA
GTACCGTGTGTTAGCCCTTAAAAAGCTTCAAACTAGAGCGGCCAATTTA
GAATCCAAATTCCTGAGGGAATTTCATGACATTGAAAGAAAGTTTGCTG
AAATGTACCAACCCTTACTGGAAAAAAGACGTCAGATCATCAATGCAAT
CTATGAACCTACAGAAGAGGAATGTGAATATAAATCAGACTCTGAGGAC
TGTGATGATGAGGAAATGTGTCATGAAGAGATGTATGGTAATGAGGAGG
GTATGGTACATGAATATGTGGATGAGGACGATGGTTATGAGGACTATTA
TTATGATTATGCTGTGGAAGAGGAGGAGGAGGAGGAGGAGGAGGACGA
CATTGAGGCTACTGGAGAAGAGAATAAAGAAGAGGAGGATCCTAAGGG
AATTCCTGATTTTTGGCTAACTGTTTTAAAAAACGTTGATACACTCACTC
CTTTGATTAAGAAATATGATGAGCCTATTCTGAAGCTCCTGACAGATATT
AAAGTTAAGCTTTCAGATCC

FIG. 9 cont.



TGGCGAGCCCCTCAGTTTCACACTAGAATTTCACTTCAAACCCAATGAAT
ATTTCAAAAATGaGTTGTTGACAAAGACCTATGTGCTGAAGTCAAAGCTA
GCATATTATGATCCCCATCCCTATAGGGGAACTGCGATTGAGTATTCCAC
AGGCTGTGAGATAGATTGGAATGAAGGAAAGAATGTCACTTTGAAAACC
ATCAAGAAGAAACAGAAACATCGGATCTGGGGAACAATCCGAACTGTAA
CTGAAGATTTTCCCAAGGATTCATTTTTCAATTTTTTCTCTCCTCATGGAA
TCACCTCAAATGGAAGGGATGGAAATGATGATTTTTTACTTGGTCACAAT
TTACGTACTTACATAATTCCAAGATCAGTATTATTTTTCTCAGGTGATGCA
CTGGAATCTCAGCAGGAGGGGGTAGTTAGAGAAGTTAATGATGCAATTT
ATGACAAAATTATTTATGATAATTGGATGGCTGCAATTGAGGAAGTTAAA
GCTTGTTGCAAAAACCTTGAGGCATTAGTAGAAGACATTGATCGTTAGA
GCAGAGTATACATGGCCCTGAAATTAACTgCCCTAGATATAGTTACTCAA
GGTATAAGAAgCCTTGTGTTCTGTATTTTgCTTTGTAGTGTTAGTTAAAAC
ATATGTTTCAAAAATATAAGAAAAGTTCAAAAACTAATTAATTTGACCTT
GAGTTTTAGTAGTAGAATGTTTTCAAGAAATGTACACTGTGGTAAATGAT
TTAAAACACTAGTATAGTGTTGTGTAGCTTAATCCTTCTGAAGTCTTTTTG
TCATGTAGCTATTAATCTGTGGCTATGAAATGATCAGAAATGCTAAGTGA
GATCAATATTTGTTTGGAAAAAAAATCTTGGGAAACAACCCAAGGGTTTT
CGCTGTTGTTGTTTTTCTTTTTCTATTTTTGTTTACTTAGTCCTTTAGCTAG
TGGATTTAATTTTGTTGTGCCTGCTTCATTTTGCAATAACAATGCAGTAG
AATTTAAAACTTGGATGCTTAAGAGGCCTGCATATAGATAAGAATTTCAG
GCAAAACTACATTTATTGTTAATAACAGCTTGTTCATAGGCTCTTGTATTT
TATGTAACTGTGATAAATAATGAAAACTTAGTTATATTGAGGTTATTGTT
TGTCGGTGAAGTGTTAGTCACAGTATTTTCAAAAGTTTGCACATATTGTT
CTGTGTAATTGTGTAAGCCATAATTACAGTGTTTAATTCTCTTTTCCTATT
ACATCATTCATTGAAAGTGATCACTTTACCATTTTGAAAAGATATTTCGT
GTTCTTTCACTGCAAAATAAAAAGAATAAAAATTTCAGAGTGTCTCATGG
AATTCC

[SEQ ID NO:3]

FIG. 10

human *BPX* 5' region

ACTTAAAGGAAAAATTTATCTATAAACTGACAGAATTTAGAAATAAATA
CAACAATATGTAAACAGTTTTAATATCTGTGATAGTAACAAATTCTTTAA
ATCTGGAAAATAATAGTCACTTAAAATTTTAAAAAATTGTTCAATTAATA
AATGATCCAAGTTAGAAATATGAACAAAATAAACCTCACCAATAATTAC
TATAGAGAGGAAATTTTAATTACTGCAAAGCTTTCCATCCTATAAATACA
TTATCAAATAGTTTAACCATTTCTTTAATGCTGAGATTTAGATTATTTCCA
ATTAACTCAAAAGCATCAAGCAAATGTTATGATTTCTAAGAATAAACATA
ACTTTCCATTTTGGCTTTTGTATATATGTATATTTCTAACGGCTGTTAAAG
CCAGCATTAAGAAGGAGAAGCAGAAAGTCAGTATTGGGACTGGGGTTAT
TTATAAGCCAGGCAACTGGTTAATTGTGGTTAATTGTCTGGTATGTTTAC
TAGTCACGTAGTTGTATACACCATACTAGTTTTTCATCACAGGCCCTCAT
TCGCCCCCACTGCCATCGGACTTCCTCCTCCTCCCCTCACAGGAAATGTT
TCGAGAATTTTTCAACCTAAAATCATATAGCTTGTGAAAAATACCGACAA
ACATAATATAGAATATTTAAATAACTGACACGCCACCTAAAGACCATCA
GTGCTAATTCCTGGTGTTTTTAATCTTTGAAGCGTTTGTTTATCAGCTCTT
CCACCATCCACCTCTCCCCTCCCCAGGTCCCCGATCTAAAATCAAAGAG
ATTGATTTAGGATGGGTGGGTGCCTTGTCTTCTCTCATTGTTCGACATTTT
AGTTACGTTTTCTCTGAGCTCTCTGGAAAGCATAAAAGTATAATATCTGT
TAAAAGTTGGATGAATGAACTAATGAACGCAATGGGATTCCAGAAAACT
CTGCGGGAGATGGGCTAGAGGACGAGGAGGAGGTGGATGAATCAGCCA
TGTTAGAGAGCCTGGGAAGGTGAGCAGAGTTGAAAACTTGATAG
ATCTAATAATTTACTGGCTCTGGGTTTGTCAGTCACTACATTGCAGCAAA
TGAGATTAGAGCATAGTTGTGGGAGGGAAGGAGGTGACGCAGCAATCTA
TTTGCACCTAGAAATTTTAGGCAAGTGATAGCTGCGTAATCATACTGCGG
CACCGTTTTTTTCTTGCAGCAGTAGCTGCTTGCGGAGGAGGTCTGCCCAC
TGCAGCTCTCTGCAGTCTCCGGCTCTCTCCTGCAGGATCGGTCAACGCAG
CCGTCGCCGCCCTCTGCACCCAGCCCAGGTCGCCACTGCTTCAGTCCGGT
TCTCAAAGCCTCAGCACCATCTTTTATCCCCGAGCAGCCTGGATCGTCGT
TCCCTCAGTCCGGACGCCACTGCTAGGTCCGACCACCGCCGCTTCTGATA
TTTCGGTGAGTCTTTTCCTGTGGAGGTTTGGTCTCCCGATCTCTGTGGTA
GCCACCTTAGGCGTGTACGGTCCTTTGAAAA

[SEQ ID NO:4]

genes[5], genes encoding cell adhesion molecules like cadherins[6], and genes affecting neural cell division, such as *p53* and *Nf1*[7], have all been implicated in the process of neurulation.

One family of genes, which has been implicated in the control of mitotic events[8,9], is the *NAP-1* family. The NAP-1 protein was first identified in Xenopus lævis[10] and homologous proteins subsequently isolated from drosophila[11], yeast[12] and man[9]. NAP-1 and NAP-1 like proteins have been shown to transfer nucleosome units to naked DNA[10], to stimulate transcription factor binding to nucleosomal DNA[13], and to act as core histone shuttle implicated in the transport histones from the cytoplasm to the nucleus[14]. Control of mitotic events may depend on the role of NAP-1 and NAP-1 like proteins in chromatin assembly and remodeling or more directly through their binding to cyclins, which is mediated by a domain also found in the tumor associated SET proteins[15].

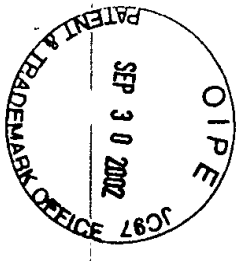
The recently isolated murine X-linked *Nap1l2 (Bpx)* and its human homologue *NAP1L2 (BPX)* have a highly restricted pattern of expression, being expressed exclusively in the nervous system[16]. In this respect, *NAP1L2* and the X-linked brain-specific *NAP1L3*[17] differ from the ubiquitously expressed *NAP1L1* and *NAP1L4* genes. The limited expression pattern of these genes suggests a particular and specialized function, possibly through an effect on nucleosome assembly or cell cycle regulation, specific to neural function.

Gene targeting techniques can be used to introduce therapeutic polynucleotides, e.g. naturally occurring unmutated, *Napl12 and NAP1L2* genes, into a host cell containing a mutated *Napl12* or *NAP1L2* gene. One of the preferred targeting techniques according to the present invention consists of a process for specific replacement, such as the DNA targeting technique described in PCT patent application N° WO 90/11354 (Institut Pasteur), incorporated herein by reference. Such a DNA targeting process makes it possible to insert the therapeutic nucleotide according to the invention behind an endogenous promoter, which has the desired functions (for example, specificity of expression in the selected target animal or embryo).

Absence of NAP1L2 protein (especially due to mutations of the corresponding genes or of their promoters) leads to overproduction of neural cells; expression of NAP1L2 or subfragments or derivatives in cells (neural/tumors/others) can prevent further proliferation and then can be used as a therapy. On the contrary, modification of Napl1L2/NAP1L2 expression (especially due to mutations of these genes or inefficiency of their promoters) leads to over production of neural cells and thereby allow regeneration or survival of neurons and therefore use as a therapy.

(SEQ ID NO. 6)

Genomic sequence *BPX* human



1. acttaaaggaaaaatttatctataaactgacagaatttagaaataaatacaacaatatgtaaacagttttaatatctgtg
2. atagtaacaaattctttaaatctggaaaataatagtcacttaaaatttttaaaaaattgttcaattaataaatgatccaag
3. ttagaaatatgaacaaaataaacctcaccaataattactatagagaggaaatttttaattactgcaaagctttccatccta
4. taaatacattatcaaatagtttaaccatttctttaatgctgagatttagattatttccaattaactcaaaagcatcaagc
5. aaatgttatgatttctaagaataaacataacttttccatttttggctttttgtatatatgtatatttctaacggctgttaaag
6. ccagcattaagaaggagaagcagaaagtcagtattgggactggggttatttataagccaggcaactggttaattgtggtt
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20. ATGGCCGAGTCAGAGAACCGCAAGGAGCTGTCAGAATCCAGTCAAGAAGAGGCTGGTAATCAGATAATGGTGGAAGGGCT
21. CGGGGAACATCTGGAGCGCGGTGAAGATGCCGCTGCTGGGCTTGGAGACGATGGGAAGTGCGGTGAAGAAGCTGCCGCTG
22. GGCTTGGGGAAGAAGGGGAAAACGGTGAAGATACTGCTGCTGGGTCCGGGGAAGATGGGAAAAAAGGTGGCGATACTGAT
23. GAGGACTCAGAGGCAGACCGTCCAAAAGGACTTATCGGTTATGTTTTAGATACAGACTTTGTTGAAAGTCTACCTGTGAA
24. AGTTAAGTACCGTGTGTTAGCCCTTAAAAAGCTTCAAACTAGAGCGGCCAATTTAGAATCCAAATTCCTGAGGGAATTTC
25. ATGACATTGAAAGAAGTTTGCTGAAATGTACCAACCCTTACTGGAAAAAAGACGTCAGATCATCAATGCAATCTATGAA
26. CCTACAGAAGAGGAATGTGAATATAAATCAGACTCTGAGGACTCTGATGATGAGGAAATGTGTCATGAAGAGATGTATGG
27. TAATGAGGAGGGTATGGTACATGAATATGTGGATGAGGACGATGGTTATGAGGACTATTATTATGATTATGCTGTGGAAG
28. AGGAGGAGGAGGAGGAGGAGGAGGACGACATTGAGGCTACTGGAGAAGAGAATAAAGAAGAGGAGGATCCTAAGGGAATT
29. CCTGATTTTTGGCTAACTGTTTTAAAAAACGTTGATACACTCACTCCTTTGATTAAGAAATATGATGAGCCTATTCTGAA
30. GCTCCTGACAGATATTAAAGTTAAGCTTTCAGATCCTGGCGAGCCCCTCAGTTTCACACTAGAATTTCACTTCAAACCCA
31. ATGAATATTTCAAAAATGaGTTGTTGACAAAGACCTATGTGCTGAAGTCAAAGCTAGCATTATGATCCCCATCCCTAT
32. AGGGGAACTGCGATTGAGTATTCCACAGGCTGTGAGATAGATTGGAATGAAGGAAAGAATGTCACTTTGAAAACCATCAA
33. GAAGAAACAGAAACATCGGATCTGGGGAACAATCCGAACTGTAACTGAAGATTTTCCCAAGGATTCATTTTTCAATTTTT
34. TCTCTCCTCATGGAATCACCTCAAATGGAAGGGATGGAAATGATGATTTTTTACTTGGTCACAATTTACGTACTTACATA
35. ATTCCAAGATCAGTATTATTTTTCTCAGGTGATGCACTGGAATCTCAGCAGGAGGGGGTAGTTAGAGAAGTTAATGATGC
36. AATTTATGACAAAATTATTTATGATAATTGGATGGCTGCAATTGAGGAAGTTAAAGCTTGTTGCAAAAACCTTGAGGCAT
37. TAGTAGAAGACATTGATCGTTAGAGCagagtatacatggccctgaaattaactgccctagatatagttactcaagGtata
38. agaagccttgtgttctgtattttgctttgtagtgttagttaaaacatatgtttcaaaaatataagaaaagttcaaaaact
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43. tttaaaacttggatgcttaagaggcctgcatatagataagaatttcaggcaaaactacatttattgttaataacagcttg
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45. tgttagtcacagtattttcaaaagtttgcacatattgttctgtgtaattgtgtaagccataattacagtgtttaattctc
46. ttttcctattacatcattcattgaaagtgatcactttaccattttgaaaagatatttcgtgttctttcactgcaaaataa
47. aaagaataaaaatttcaga

In re Application of: ROGNER et al.
Application No.: 09/847,665
Filed: ay 3, 2001
For: IDENTIFICATION OF NEURAL DEFECTS ASSOCIATED
WITH THE NUCLEOSOMAL ASSEMBLY PROTEIN 1l2
Attorney Docket No.: 03495-0203

PAGES OF INCORRECTLY PUBLISHED APPLICATION

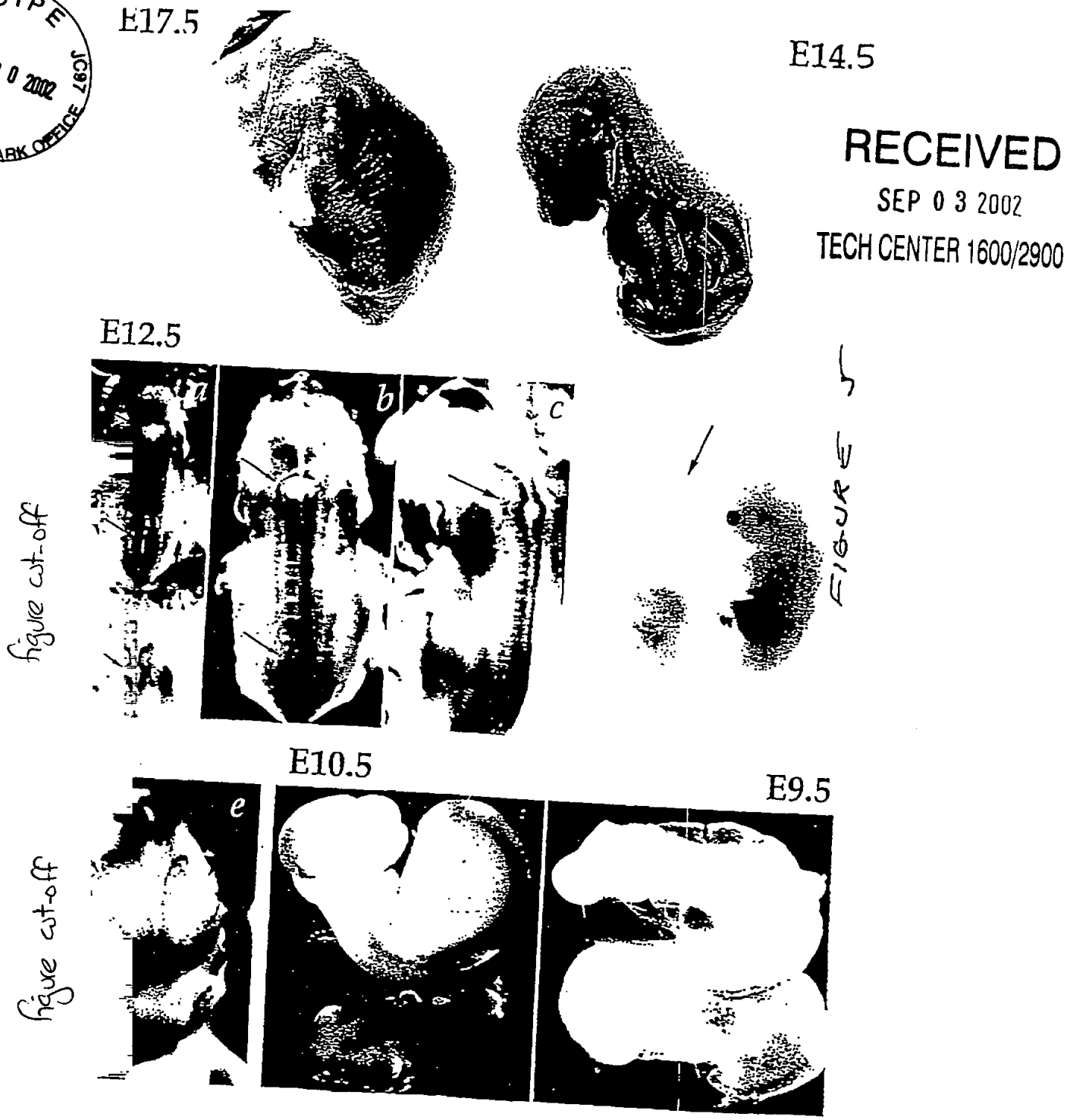

FIG. 7

Sequence clone *Bpx* promoter murin SpeI-SalI fragment

ACTAGTCATATAGCTGGCTCTTTTACAAAAGGCTTCAACACCCCTCCCCC
CACACTTTAGTCATCCGTCATCTCTTCCTCATCAGGAAATATTATGAGAA
TTTTCCCATTTAAAATCACACAGGTTGTGAAAATTACAGAAACCAGGGTA
CAGAATATTTAAACCACTGTCAGTTACATCATCCAAAGGCCACCTATGCT
TATTTTTGGTAATTTTAAACCTCAAAGGATCTCTTTGTGGGCTCCTCCACT
ACCCTCCTCTCTTTCCCAGAGCCTCAGGTTATAACCAAAGGGATAGACTA
AAGACAATCCAGTACCTTGCCCATTTTTTTCATTCCTTGTCACTGTTTCCA
TATAGCTCTTTTGAAATTATGAACATATAGTATCAGTTGAAAACGGAATG
AATGATACTGCATTTCTGCAAAATTCCACAGGCTATAGGGTGGAAGATG
AGCCATAGGTGGAGGAATCAGCCATATTAGAGAATCTGGGAAGGCAAG
AGGTGTTGAAATTTTGATTCATCTACTAATTTACTGGCTCAGGATTTGTC
AATCACTGCAGCCTGGCAAATGAGATTAGAGAAGAGTCCTGGGAGGGA
AGGGGTGACGCAGCAACCTGCATACACTTAAAAAAAAAAGAGCTGAGAG
ACAACTGCGTAATCATACTGCGGCACCAGTTCCTCCATCCCTCCGCCCCC
GAGTGGCTGGAGCAGCTGCTTGCGGAGGTCTGCCCACTGCGGCTCTCTG
CAGTCTCTAGCCTGTTCCTTCAGGGCCTAGAGTCTCCGCCCAGACAGCCG
GTTTCAATTCTGCTATCCCAGCTTCAGCACCGTCTTTTATACTGCTTGCTG
CCTGCCATCAGTGCAGCCGCCGCCGCCTCTTGGTTCATCTCTGCCAGATC
ATCGCGCATCTGCTGTATTGGTGAGTCTTCCTGCGGAGGTCAGGTCTCCT
GATCTGCGGGCTTAGCCACCATAAGTGCAGGCGATCGTTTGAAAACAAT
GGCTGAATCAGTCGACCTCGAGGGGGGGCGTACCTTGCCCATTTTTTTCA
TTCCTTGTCACTGTTTCCATATAGCTCTTTTGAAATTATGAACATATAGTA
TCAGTTGAAAACGGAATGAATGATACTGCATTTCTGCAAAATTCCACAG
GCTATAGGGTGGAAGATGAGCCATAGGTGGAGGAATCAGCCATATTAGA
GAATCTGGGAAGGCAAGAGGTGTTGAAATTTTGATTCATCTACTAATTTA
CTGGCTCAGGATTTGTCAATCACTGCAGCCTGGCAAATGAGATTAGAGA
AGAGTCCTGGGAGGGAAGGGGTGACGCAGCAACCTGCATACACTTAAA
AAAAAAGAGCTGAGAGACAACTGCGTAATCATACTGCGGCACCAGTTCC
TCCATCCCTCCGCCCCCGAGTGGCTGGAGCAGCTGCTTGCGGAGGTCTG
CCCACTGCGGCTCTCTGCAGTCTCTAGCCTGTTCCTTCAGGGCCTAGAGT
CTCCGCCCAGACAGCCGGTTTCAATTCTGCTATCCCAGCTTCAGCACCGT
CTTTTATCCCCACTGCTTGCTGCCTGCCATCAGTGCAGCCGCCGCCGCCT
CTTGGTTCATCTCTGCCAGATCATCGCGCATCTGCTGTATTGGTGAGTCT
TCCTGCGGAGGTCAGGTCTCCTGATCTGCGGGCTTAGCCACCATAAGTG
CAGGCGATCGTTTGAAAACAATGGCTGAATCAGTCGAC

[SEQ ID NO: 1]

FIG. 8 cont.

CGTACTTACATAATTCCAAGATCAGTGTTATTTTTCTCAGGAGATGCACT
TGAATCTCAGCAGGAGGGTGTAGTTAGGGAAGTTAATGACGAAATATAT
GACAAAATTATTTATGATGATTGGATGGCTGCAATTGAAGAGGTTAAAG
CCTGTTGCAAAAATCTTGAGGCATTAGTAGAAGATATTGATCGTTAAAAC
AGAGTAGATGCTTTTGAAACTAACTGCTCTACATGCAGTTACTGAAGACA
TAAGCAGTTAATATTGTCTTGTGTTCTGCATTTTTTCCTGTCATGCCAGTT
TAAAAATTCAAATACTAATTAATCTGACCTTGCATTGTAGTGGTATGATG
TTTTCAAGACATGTAGACTGTGATAAATGATTAAGACATTAATAGTCTGT
AGTATAACCCTTCTGAAGTCCTTGTGCCATGTATCTATTAATCTGTGGCT
GTGAATATTATTAGAAGTGCTAAATGAGATTATTTGTTTGCAAAGAAAAT
ATTGGAAACCTACCTAAGAGTGCTTTGCTATTTTCCCCCTTATCCTCTTAG
TGCTTTGGCCAATTGACTTTATTGTGCCTGCTTCATTTTGCAGTAAATATG
CAGTAGAATTTAAAACTTGAATGCCTAAGAGGCCTGCATATGATTGAGA
ATTTCAGGCAAAATCATATTTATTATTGATAACAGCTAGTGCAAGGCTTC
TGATTGTATGTGACTGTGATAAATAATAAAACTCAATTGTATTGAAGTTA
CTGTTTATCATTGACATGTGAGTTACAGTATTTTCAAATGTTGCAAATATT
GTCCTGTGTAATTGTGTAAACTGTGATTACAGTGTACATTTTTTTCATAAT
ATACTGAATCATTCATTGAAATGGACACTTTACCATTTCTGAAAATACAT
TTCATATTCTGTTCATTCACTGAAAAATAAAATGAATAAAAATTT

[SEQ ID NO: 2]

FIG. 9

***BPX* human cDNA identical to genomic DNA**

TGTTAGAGAGCCTGGGAAGGTGAGcAGAGcTGAAAACTTGATAGATCTA
ATAATTTACTGGCTCTGGGTTTGTCAGTCACTACATTGCAGCAAATGAGA
ITAGAGCATAGTTGTGGGAGGGAAGGAGGTGACGCAGCAATCTATTTGC
ACCTAGAAATTTTAGGCAAGTGATAGCTGCGTAATCATACTGCGGCACC
GTTTTTTTCTTGCAGCAGTAGCTGCTTGCGGAGGAGGTCTGCCCACTGCA
GCTCTCTGCAGTCTCCGGCTCTCTCCTGCAGGATCGGTCAACGCAGCCGT
CGCCGCCCTCTGCACCCAGCCCAGGTCGCCACTGCTTCAGTCCGGTTCTC
AAAGCCTCAGCACCATCTTTTATCCCCGAGCAGCCTGGATCGTCGTTCCC
TCAGTCCGGACGCCACTGCTAGGTCCGACCACCGCCGCTTCTGATATTTC
GGTGAGTCTTTTCCTGTGGAGGTTTGGTCTCCCGATCTCTGTGGTAGCCA
CCTTAGGCGTGTACGGTCCTTTGAAAAATGGCCGAGTCAGAGAACCGCA
AGGAGCTGTCAGAATCCAGTCAAGAAGAGGCTGGTAATCAGATAATGGT
GGAAGGGCTCGGGGAACATCTGGAGCGCGGTGAAGATGCCGCTGCTGG
GCTTGGAGACGATGGGAAGTGCGGTGAAGAAGCTGCCGCTGGGCTTGG
GGAAGAAGGGGAAAACGGTGAAGATACTGCTGCTGGGTCCGGGGAAGA
TGGGAAAAAAGGTGGCGATACTGATGAGGACTCAGAGGCAGACCGTCC
AAAAGGACTTATC
GGTTATGTTTTAGATACAGACTTTGTTGAAAGTCTACCTGTGAAAGTTAA
GTACCGTGTGTTAGCCCTTAAAAAGCTTCAAACTAGAGCGGCCAATTTA
GAATCCAAATTCCTGAGGGAATTTCATGACATTGAAAGAAAGTTTGCTG
AAATGTACCAACCCTTACTGGAAAAAAGACGTCAGATCATCAATGCAAT
CTATGAACCTACAGAAGAGGAATGTGAATATAAATCAGACTCTGAGGAC
TGTGATGATGAGGAAATGTGTCATGAAGAGATGTATGGTAATGAGGAGG
GTATGGTACATGAATATGTGGATGAGGACGATGGTTATGAGGACTATTA
TTATGATTATGCTGTGGAAGAGGAGGAGGAGGAGGAGGAGGAGGACGA
CATTGAGGCTACTGGAGAAGAGAATAAAGAAGAGGAGGATCCTAAGGG
AATTCCTGATTTTTGGCTAACTGTTTTAAAAAACGTTGATACACTCACTC
CTTTGATTAAGAAATATGATGAGCCTATTCTGAAGCTCCTGACAGATATT
AAAGTTAAGCTTTCAGATCC

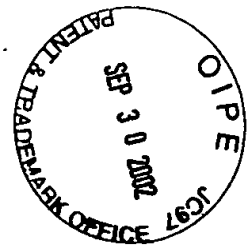

FIG. 9 cont.

TGGCGAGCCCCTCAGTTTCACACTAGAATTTCACTTCAAACCCAATGAAT
ATTTCAAAAATGaGTTGTTGACAAAGACCTATGTGCTGAAGTCAAAGCTA
GCATATTATGATCCCCATCCCTATAGGGGAACTGCGATTGAGTATTCCAC
AGGCTGTGAGATAGATTGGAATGAAGGAAAGAATGTCACTTTGAAAACC
ATCAAGAAGAAACAGAAACATCGGATCTGGGGAACAATCCGAACTGTAA
CTGAAGATTTTCCCAAGGATTCATTTTTCAATTTTTTCTCTCCTCATGGAA
TCACCTCAAATGGAAGGGATGGAAATGATGATTTTTTACTTGGTCACAAT
TTACGTACTTACATAATTCCAAGATCAGTATTATTTTTCTCAGGTGATGCA
CTGGAATCTCAGCAGGAGGGGGTAGTTAGAGAAGTTAATGATGCAATTT
ATGACAAAATTATTTATGATAATTGGATGGCTGCAATTGAGGAAGTTAAA
GCTTGTTGCAAAAACCTTGAGGCATTAGTAGAAGACATTGATCGTTAGA
GCAGAGTATACATGGCCCTGAAATTAACTgCCCTAGATATAGTTACTCAA
GGTATAAGAAgCCTTGTGTTCTGTATTTTgCTTTGTAGTGTTAGTTAAAAC
ATATGTTTCAAAAATATAAGAAAAGTTCAAAAACTAATTAATTTGACCTT
GAGTTTTAGTAGTAGAATGTTTTCAAGAAATGTACACTGTGGTAAATGAT
TTAAAACACTAGTATAGTGTTGTGTAGCTTAATCCTTCTGAAGTCTTTTTG
TCATGTAGCTATTAATCTGTGGCTATGAAATGATCAGAAATGCTAAGTGA
GATCAATATTTGTTTGGAAAAAAAATCTTGGGAAACAACCCAAGGGTTTT
CGCTGTTGTTGTTTTTCTTTTTCTATTTTTGTTTACTTAGTCCTTTAGCTAG
TGGATTTAATTTTGTTGTGCCTGCTTCATTTTGCAATAACAATGCAGTAG
AATTTAAAACTTGGATGCTTAAGAGGCCTGCATATAGATAAGAATTTCAG
GCAAAACTACATTTATTGTTAATAACAGCTTGTTCATAGGCTCTTGTATTT
TATGTAACTGTGATAAATAATGAAAACTTAGTTATATTGAGGTTATTGTT
TGTCGGTGAAGTGTTAGTCACAGTATTTTCAAAAGTTTGCACATATTGTT
CTGTGTAATTGTGTAAGCCATAATTACAGTGTTTAATTCTCTTTTCCTATT
ACATCATTCATTGAAAGTGATCACTTTACCATTTTGAAAAGATATTTCGT
GTTCTTTCACTGCAAAATAAAAAGAATAAAAATTTCAGAGTGTCTCATGG
AATTCC

[SEQ ID NO:3]

FIG. 10

human *BPX* 5' region

ACTTAAAGGAAAAATTTATCTATAAACTGACAGAATTTAGAAATAAATA
CAACAATATGTAAACAGTTTTAATATCTGTGATAGTAACAAATTCTTTAA
ATCTGGAAAATAATAGTCACTTAAAATTTTAAAAAATTGTTCAATTAATA
AATGATCCAAGTTAGAAATATGAACAAAATAAACCTCACCAATAATTAC
TATAGAGAGGAAATTTTAATTACTGCAAAGCTTTCCATCCTATAAATACA
TTATCAAATAGTTTAACCATTTCTTTAATGCTGAGATTTAGATTATTTCCA
ATTAACTCAAAAGCATCAAGCAAATGTTATGATTTCTAAGAATAAACATA
ACTTTCCATTTTGGCTTTTGTATATATGTATATTTCTAACGGCTGTTAAAG
CCAGCATTAAGAAGGAGAAGCAGAAAGTCAGTATTGGGACTGGGGTTAT
TTATAAGCCAGGCAACTGGTTAATTGTGGTTAATTGTCTGGTATGTTTAC
TAGTCACGTAGTTGTATACACCATACTAGTTTTTCATCACAGGCCCTCAT
TCGCCCCCACTGCCATCGGACTTCCTCCTCCTCCCCTCACAGGAAATGTT
TCGAGAATTTTTCAACCTAAAATCATATAGCTTGTGAAAAATACCGACAA
ACATAATATAGAATATTTAAATAACTGACACGCCACCTAAAGACCATCA
GTGCTAATTCCTGGTGTTTTTAATCTTTGAAGCGTTTGTTTATCAGCTCTT
CCACCATCCACCTCTCCCCTCCCCAGGTCCCCGATCTAAAATCAAAGAG
ATTGATTTAGGATGGGTGGGTGCCTTGTCTTCTCTCATTGTTCGACATTTT
AGTTACGTTTTCTCTGAGCTCTCTGGAAAGCATAAAAGTATAATATCTGT
TAAAAGTTGGATGAATGAACTAATGAACGCAATGGGATTCCAGAAAACT
CTGCGGGAGATGGGCTAGAGGACGAGGAGGAGGTGGATGAATCAGCCA
TGTTAGAGAGCCTGGGAAGGTGAGCAGAGTTGAAAACTTGATAG
ATCTAATAATTTACTGGCTCTGGGTTTGTCAGTCACTACATTGCAGCAAA
TGAGATTAGAGCATAGTTGTGGGAGGGAAGGAGGTGACGCAGCAATCTA
TTTGCACCTAGAAATTTTAGGCAAGTGATAGCTGCGTAATCATACTGCGG
CACCGTTTTTTTCTTGCAGCAGTAGCTGCTTGCGGAGGAGGTCTGCCCAC
TGCAGCTCTCTGCAGTCTCCGGCTCTCTCCTGCAGGATCGGTCAACGCAG
CCGTCGCCGCCCTCTGCACCCAGCCCAGGTCGCCACTGCTTCAGTCCGGT
TCTCAAAGCCTCAGCACCATCTTTTATCCCCGAGCAGCCTGGATCGTCGT
TCCCTCAGTCCGGACGCCACTGCTAGGTCCGACCACCGCCGCTTCTGATA
TTTCGGTGAGTCTTTTCCTGTGGAGGTTTGGTCTCCCGATCTCTGTGGTA
GCCACCTTAGGCGTGTACGGTCCTTTGAAAA

[SEQ ID NO:4]

IDENTIFICATION OF NEURAL DEFECTS ASSOCIATED WITH THE NUCLEOSOMAL ASSEMBLY PROTEIN 112 GENE

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is based on and claims the benefit of U.S. Provisional Application Ser. No. 60/202,111, filed May 5, 2000 (attorney docket no. 03495.6048) The entire disclosure of this application is relied upon and incorporated by reference herein.

BACKGROUND OF THE INVENTION

[0002] This invention relates to the discovery of a link between defects in development of the central nervous system of a mammal and mutations in a gene, which result in a loss of biological function of protein encoded by the gene. Mutated forms of the gene, the RNA, and the protein it encodes are useful in diagnosis of predisposition to genetic defects.

[0003] Neurulation is a complex process of histogenesis involving the precise temporal and spatial organization of gene expression. Amongst the molecular components necessary for neurulation are proneural genes determining primary cell fate, neurogenic genes involved in the lateral inhibition pathway, and genes controlling the frequency of mitotic events[1,2]. This underlying complexity is reflected in the aetiology and genetics of human neural tube defects (NTDs), which are of both multifactorial and multigenic origin[3]. Similar complexity is observed in mouse models of NTDs where genes affecting cell fate such as Sonic hedgehog[4] and the Pax genes[5], genes encoding cell adhesion molecules like cadherins[6], and genes affecting neural cell division, such as p53 and Nfl[7], have all been implicated in the process of neurulation.

[0004] One family of genes, which has been implicated in the control of mitotic events[8,9], is the NAP-1 family. The NAP-1 protein was first identified in Xenopus laevis[10] and homologous proteins subsequently isolated from drosophila[11], yeast[12] and man[9]. NAP-1 and NAP-1 like proteins have been shown to transfer nucleosome units to naked DNA[10], to stimulate transcription factor binding to nucleosomal DNA[13], and to act as core histone shuttle implicated in the transport histones from the cytoplasm to the nucleus[14]. Control of mitotic events may depend on the role of NAP-1 and NAP-1 like proteins in chromatin assembly and remodeling or more directly through their binding to cyclins, which is mediated by a domain also found in the tumor associated SET proteins[15].

[0005] The recently isolated murine X-linked Nap112 (Bpx) and its human homologue NAP1L2 (BPX) have a highly restricted pattern of expression, being expressed exclusively in the nervous system[16]. In this respect, NAP1L2 and the X-linked brain-specific NAP1L3[17] differ from the ubiquitously expressed NAP1L1 and NAP1L4 genes. The limited expression pattern of these genes suggests a particular and specialized function, possibly through an effect on nucleosome assembly or cell cycle regulation, specific to neural function.

[0006] Neural tube defects occur with a frequency of 3.5/1000 births. There is a need in the art for the identification of genes associated with defects in development of the central nervous system. In particular, there is a need for diagnostic tests and biological materials to identify predisposition to developmental defects.

SUMMARY OF THE INVENTION

[0007] This invention aids in fulfilling these needs in the art. More particularly, this invention relates to the discovery of the role of Nap112 in mouse development. A targeted deletion of the X-linked Nap112 gene in male ES cells, which would be expected to lead to the complete absence of the NAP1L2 protein, was created. In close agreement with the first detectable signs of Nap112 expression at day E9.5, the mutation resulted in embryonic lethality from mid-gestation onwards. Surviving embryos derived from ES cell-morula aggregates exhibited neural tube closure defects, associated with a marked overproduction of neuronal tissue.

[0008] This invention shows that Nap112 plays an essential role in the development of the nervous system and suggests a putative role for it in the control of cell proliferation and differentiation processes. Aberrant cell cycle regulation and differentiation may, therefore, be one of the mechanisms underlying certain neural tube defects (NTDs). This invention also identifies the human NAP1L2 gene as a gene for certain X-linked and spontaneous forms of these disorders.

[0009] One embodiment of this invention relates to a method for screening neural system defects in the mammal and especially in the human. The method comprises: (A) providing genomic material from the human; (B) detecting a modification of the NAP1L2 gene in the genomic material, wherein the modification is selected from a) substitution, b) deletion, c) frame-shift, d) insertion aberent or e) altered epigenetic control that causes a loss of biological function in the NAP1L2 gene; and (C) corre-lating the modification of the gene with a potential for a neural system defect. In a preferred embodiment, the modification in the NAP1L2 gene is detected by hybridization with a labeled probe, such as a probe. of SEQ ID NO:3 or a fragment thereof. The modification can be detected, for example, by (A) amplification of the genomic material using PCR; (B) sequencing the material to detect the modification of the nucleotide sequence; and (C) correlating the modification of the gene with a potential for neural system defects. The modification can be detected by quantification of the transcript using PCR or Northern Blot.

[0010] In another embodiment, the invention provides a method for screening neural system defects in a human, this method comprises: (A) providing biological material from the human; (B) detecting the absence, inappropriate, or modified expression of NAP1L2 gene product using labeled antibodies to the gene product; and (C) correlating the absence, inappropriate, or modified expression with a potential for neural system defects. The antibodies can be polyclonal or monoclonal.

[0011] The neural system defect can result from a failure of, or incomplete, neural tube closure, incomplete neural tube closure resulting in spina bifida, incomplete neural tube closure resulting in anencephaly, neural system defect relating to an inappropriate proliferation of surface ectoderm-derived cells, neural defect resulting in a loss of brain structure, neural system defect resulting from disorganiza-

desired functions (for example, specificity of expression in the selected target animal or embryo).

[0133] Absence of NAP1L2 protein (especially due to mutations of the corresponding genes or of their promoters) leads to overproduction of neural cells; expression of NAP1L2 or subfragments or derivatives in cells (neural/tumors/others) can prevent further proliferation and then can be used as a therapy. On the contrary, modification of Nap1l2/NAP1L2 expression (especially due to mutations of these genes or inefficiency of their promoters) leads to over production of neural cells and thereby allow regeneration or survival of neurons and therefore use as a therapy.

[0134] The following plasmids were deposited at the Collection National de Cultures de Microorganismes (C.N.C.M.), of Institut Pasteur, 28 rue due Docteur Roux, F-75724 Paris, Cedex 15, France, and assigned the following Accession Nos.:

PLASMID	DEPOSIT DATE	ACCESSION NO.
pCUR1-2	April 25, 2000	I-2463
BPX-1	April 25, 2000	I-2464
BPX-2	April 25, 2000	I-2465
BPX-3	April 25, 2000	I-2466.

[0135] Another aspect of the invention is an eukaryotic cell containing the insert contained in the plasmid BPX-1 or BPX-2 or BPX-3 or polynucleotides hybridizing under stringent conditions with the said insert.

[0136] This invention will now be described in greater detail in the following Examples.

EXAMPLE 1

Expression Profile Analysis During Mouse Development

[0137] In order to obtain a more precise overview of the profile of Nap1l2 expression, RNA in situ hybridization was performed using a Nap1l2 specific oligonucleotide probe on sections of adult mouse brain and mouse embryos corresponding to days E5.5 through to E18.5 (**FIG. 1**).

[0138] Nap1l2 expression was found throughout the nervous system, in structures belonging to both the central and peripheral nervous systems. Expression was first detectable at day E10.5, and correlates with the initial wave of neuronal differentiation (**FIG. 1**). Embryonic stages E10.5 through to E18.5 revealed that Nap1l2 expression, although predominantly in the spinal cord, was also present throughout the brain and ganglia. In the adult brain, all regions were labeled, although variation in the intensity of the labeling suggested some heterogeneity in expression levels. Signals were particularly strong in the anterior olfactory nucleus, the hippocampus, the hypothalamus and the cerebellum. The strongest Nap1l2 signal was detected in the mammillary bodies (see Bregma −2.9, **FIG. 1**).

[0139] Differentiated regions within the nervous system exhibited strong labeling, whereas ventricular zones did not show specific signals. No Nap1l2 transcripts could be detected in glial cells or in tissues other than the nervous system. Expression of Nap1l2 is likely to be restricted to post-mitotic neurons.

EXAMPLE 2

The Role of Nap1l2 Defined by Deletion Analysis Targeted Deletion and Differentiation of ES Cells

[0140] In order to establish the role of Nap1l2 we created a null mutation of the Nap1l2 gene in male ES cells, hemizygous for Nap1l2, by homologous recombination. In the knockout construct, the intronless Nap1l2 gene was partially deleted and replaced by a β-galactosidase reporter and neomycin resistance gene (**FIG. 2**). The resulting fusion protein has potential for Nap1l2 function, since it includes only five amino acids from the N-terminal end of NAP1L2, all the non-deleted C-terminal sequences being out of frame. Two targeted cell lines, 5b17 and 8b21, in which the endogenous X-linked Nap1l2 gene had been replaced by homologous recombination, were obtained (**FIG. 2**). The absence of a Nap1l2 transcript in these ES clones was confirmed by RT-PCR, and the karyotype of the clones verified on mitotic spreads.

[0141] As ES cells have the potential to differentiate into neurons in vitro, an investigation was made to determine whether the deletion of Nap1l2 affects the in vitro development of neurons. In vitro differentiation experiments are based on the formation of embryoid bodies in suspension culture. Re-attachment of the embryoid bodies after four days of culture leads to the formation of various types of differentiated cells. Formation of neurons can be induced by the addition of retinoic acid to the medium[18]. To visualize the specific cell types formed, antibodies directed against various neuronal marker proteins: nestin, which is present in precursor cells, β-tubulin III in early neurons, NF200 in differentiated neurons, and GFAP in glial cells, were used.

[0142] All three cell lines, the original ES cell line CK35 and the two recombinant ES cell lines, 5b17 and 8b21, were able to form neurons the presence of retinoic acid. In both the normal and mutant cell lies, nestin positive cells were observed two or three days after attachment of the embryoid bodies. Neurons together with glial cells usually appeared four to six days after embryoid body replating. The number of neurons developing was dependent on the concentration of retinoic acid used[19]. Whereas cultures without retinoic acid produced only a few neuronil cells, their number was substantially increased by adding 3×10^{-3} M retinoic acid.

[0143] In the absence of retinoic acid, the CK35 cell line produced as expected, only few neuronal cells. In contrast, the mutant cell lines produced large numbers of nestin positive neuronal cells increasing from about 50 cells per mm^2 one day to 200 cells per mm^2 three days after re-attachment (**FIGS. 3*a*, *b***). Many of these nestin positive cells were lacZ positive (**FIG. 3*c***). Pulse chase experiments using BrdU confirmed that these lacZ expressing cells represent a growing cell population (data not shown).

[0144] These experiments show that the Nap1l2 mutation affects the proliferation of neuronal precursor cells in vivo as well as in vitro. The dual effect of RA on both neuronal cell differentiation and G1 arrest of cell division[13] probably leads to the suppression of the proliferative effect of the Nap1l2 mutation.

EXAMPLE 3

Phenotypical Observation of Chimeras

[0145] In order to examine more closely the effect of the Nap1l2 deletion on mouse development, changes in the

[0219] 36. Gardner, R. L. Mouse chimeras obtained by the injection of cells into the blastocyst. *Nature* 220, 596-7 (1968).

[0220] 37. Wood, S. A., Pascoe, W. S., Schmidt, C., Kemler, R., Evans, M. J. & Allen, N. D. Simple and efficient production of embryonic stem cell-embryo chimeras by coculture. *Proc. Natl. Acad. Sci. USA* 90, 4582-5 (1993).

[0221] 38. Nagy, A., Rossant, J., Nagy, R., Abramow-Newerly, W. & Roder, J. C. Derivation of completely cell culture-derived mice from early-passage embryonic stem cells. *Proc. Natl. Acad. Sci. USA* 90, 8424-8 (1993).

[0222] 39. Papenbrock, T., Peterson, R. L., Lee, R. S., Hsu, T., Kuroiwa, A. & Awgulewitsch, A. Murine Hoxc-9 gene contains a structurally and functionally conserved enhancer. *Dev. Dyn.* 12, 540-7 (1998).

[0223] 40. Hogan, B., Beddington, R., Costantini, F. & Lacy, E. in *Manipulation the mouse embryo* 373-375 (ColdSpring Harbor Laboratory Press New York 1994).

move to end of claim 45

(SEQ ID NO. 6)
Genomic sequence BPX human

1. acttaaaggaaaaatttatctataaactgacagaatttagaaataaatacaacaatatgtaaacagttttaatatctgtg
2. atagtaacaaattctttaaatctggaaaataatagtcacttaaaattttaaaaaattgttcaattaataaatgatccaag
3. ttagaaatatgaacaaaataaacctcaccaataattactatagagaggaaattttaattactgcaaagctttccatccta
4. taaatacattatcaaatagtttaaccatttctttaatgctgagatttagattatttccaattaactcaaaagcatcaagc
5. aaatgttatgatttctaagaataaacataactttccattttggcttttgtatatatgtatatttctaacggctgttaaag
6. ccagcattaagaaggagaagcagaaagtcagtattgggactggggttatttataagccaggcaactggttaattgtggtt
7. aattgtctggtatgtttactagtcacgtagttgtatacaccatactagtttttcatcacaggccctcattcgcccccact
8. gccatcggacttcctcctcctcccctcacaggaaatgtttcgagaatttttcaacctaaaatcatatagcttgtgaaaaa
9. taccgacaaacataatatagaatatttaaataactgacacgccacctaaagaccatcagtgctaattcctggtgttttta
10. atctttgaagcgtttgtttatcagctcttccaccatccacctctcccctccccaggtccccgatctaaaatcaaagagat
11. tgatttaggatgggtgggtgccttgtcttctctcattgttcgacattttagttacgttttctctgagctctctggaaagc
12. ataaaagtataatatctgttaaaagttggatgaatgaactaatgaacgcaatgggattccagaaaactctgcgggagatg
13. ggctagaggacgaggaggaggtggatgaatcagccatgttagagagcctgggaaggtgagcagagttgaaaacttgatag
14. atctaataatttactggctctgggtttgtcagtcactacattgcagcaaatgagattagagcatagttgtgggagggaag
15. gaggtgacgcagcaatctatttgcacctagaaattttaggcaagtgatagctgcgtaatcatactgcggcaccgtttttt
16. tcttgcagcagtagctgcttgcggaggaggtctgcccactgcagctctctgcagtctccggctctctcctgcaggatcgg
17. tcaacgcagccgtcgccgccctctgcacccagcccaggtcgccactgcttcagtccggttctcaaagcctcagcaccatc
18. ttttatccccgagcagcctggatcgtcgttccctcagtccggacgccactgctaggtccgaccaccgccacttctgatat
19. ttcggtgagtcttttcctgtggaggtttggtctcccgatctctgtggtagccaccttaggcgtgtacggtcctttgaaaa
20. ATGGCCGAGTCAGAGAACCGCAAGGAGCTGTCAGAATCCAGTCAAGAAGAGGCTGGTAATCAGATAATGGTGGAAGGGCT
21. CGGGGAACATCTGGAGCGCGGTGAAGATGCCGCTGCTGGGCTTGGAGACGATGGGAAGTGCGGTGAAGAAGCTGCCGCTG
22. GGCTTGGGGAAGAAGGGGAAAACGGTGAAGATACTGCTGCTGGGTCCGGGGAAGATGGGAAAAAAGGTGGCGATACTGAT
23. GAGGACTCAGAGGCAGACCGTCCAAAAGGACTTATCGGTTATGTTTTAGATACAGACTTTGTTGAAAGTCTACCTGTGAA
24. AGTTAAGTACCGTGTGTTAGCCCTTAAAAAGCTTCAAACTAGAGCGGCCAATTTAGAATCCAAATTCCTGAGGGAATTTC
25. ATGACATTGAAAGAAAGTTTGCTGAAATGTACCAACCCTTACTGGAAAAAAGACGTCAGATCATCAATGCAATCTATGAA
26. CCTACAGAAGAGGAATGTGAATATAAATCAGACTCTGAGGACTCTGATGATGAGGAAATGTGTCATGAAGAGATGTATGG
27. TAATGAGGAGGGTATGGTACATGAATATGTGGATGAGGACGATGGTTATGAGGACTATTATTATGATTATGCTGTGGAAG
28. AGGAGGAGGAGGAGGAGGAGGAGGACGACATTGAGGCTACTGGAGAAGAGAATAAAGAAGAGGAGGATCCTAAGGGAATT
29. CCTGATTTTTGGCTAACTGTTTTAAAAAACGTTGATACACTCACTCCTTTGATTAAGAAATATGATGAGCCTATTCTGAA

-continued

(SEQ ID NO. 6)
Genomic sequence BPX human

30. GCTCCTGACAGATATTAAAGTTAAGCTTTCAGATCCTGGCGAGCCCCTCAGTTTCACACTAGAATTTCACTTCAAACCCA

31. ATGAATATTTCAAAAATGaGTTGTTGACAAAGACCTATGTGCTGAAGTCAAAGCTAGCATATTATGATCCCCATCCCTAT

32. AGGGGAACTGCGATTGAGTATTCCACAGGCTGTGAGATAGATTGGAATGAAGGAAAGAATGTCACTTTGAAAACCATCAA

33. GAAGAAACAGAAACATCGGATCTGGGGAACAATCCGAACTGTAACTGAAGATTTTCCCAAGGATTCATTTTTCAATTTTT

34. TCTCTCCTCATGGAATCACCTCAAATGGAAGGGATGGAAATGATGATTTTTTACTTGGTCACAATTTACGTACTTACATA

35. ATTCCAAGATCAGTATTATTTTTCTCAGGTGATGCACTGGAATCTCAGCAGGAGGGGGTAGTTAGAGAAGTTAATGATGC

36. AATTTATGACAAAATTATTTATGATAATTGGATGGCTGCAATTGAGGAAGTTAAAGCTTGTTGCAAAAACCTTGAGGCAT

37. TAGTAGAAGACATTGATCGTTAGAGCagagtatacatggccctgaaattaactgccctagatatagttactcaaggtata

38. agaagccttgtgttctgtattttgctttgtagtgttagttaaaacatatgtttcaaaaatataagaaaagttcaaaaact

39. aattaatttgaccttgagttttagtagtagaatgttttcaagaaatgtacactgtggtaaatgatttaaaacactagtat

40. agtgttgtgtagcttaatccttctgaagtctttttgtcatgtagctattaatctgtggctatgaaatgatcagaaatgct

41. aagtgagatcaatatttgtttggaaaaaaaatcttgggaaacaacccaagggttttcgctgttgttgtttttctttttct

42. atttttgtttacttagtcctttagctagtggatttaattttgttgtgcctgcttcattttgcaataacaatgcagtagaa

43. tttaaaacttggatgcttaagaggcctgcatatagataagaatttcaggcaaaactacatttattgttaataacagcttg

44. ttcataggctcttgtattttatgtaactgtgataaataatgaaaacttagttatattgaggttattgtttgtcggtgaag

45. tgttagtcacagtattttcaaaagtttgcacatattgttctgtgtaattgtgtaagccataattacagtgtttaattctc

46. ttttcctattacatcattcattgaaagtgatcactttaccattttgaaaagatatttcgtgttctttcactgcaaaataa

47. aaagaataaaaatttcaga

What is claimed is:

1. A method for screening neural system defects in a mammal, said method comprising:

(A) providing chromosomal material from said human;

(B) detecting a modification of the NAP1L2 gene in the chromosomal material, wherein said modification is selected from a) substitution, b) deletion, c) frame-shift, d) insertion aberent or e) altered epigenetic control; that causes a loss of biological function in the NAP1L2 gene; and

(C) correlating the modification of said gene with a potential for a neural system defect.

2. A method according to claim 1 where the said screening of neural system defects concerns a human being.

3. The method of claim 1, wherein said modification in the NAP1L2 gene is detected by hybridization with a labeled probe.

4. The method of claim 3, wherein said probe is a oligonucleotide probe of SEQ ID NO:3.

5. A method of claim 1, wherein said modification is detected by

(A) amplification of the chromosomal material using PCR;

(B) sequencing said material to detect the modification of the nucleotide sequence; and

(C) correlating the modification of said gene with a potential for neural system defects.

6. A method for screening neural system defects in a human, said method comprising:

(A) providing biological material from said human;

(B) detecting the absence, inappropriate, or modified expression of NAP1L2 gene product using labeled antibodies to said gene product; and

(C) correlating said absence, inappropriate, or modified expression with a potential for neural system defects.

7. The method of claim 5, wherein the said antibodies are polyclonal.

8. The method of claim 5, wherein the said antibodies are monoclonal.

9. A method of any one of claims 1 to 8, wherein the neural system defect results from a failure of or incomplete neural tube closure.

10. A method of claim 9, wherein said incomplete neural tube closure results in spina bifida.

11. The method of any one of claims 1 to 8, wherein the neural system defect results from inappropriate control of nucleosome activity in neurons.

12. The method of any one of claims 1 to 8, wherein the neural system defect results from inappropriate control of the cell cycle in neurons.

13. The method of any one of claims 1 to 7, wherein the neural system defect results from inappropriate differentiation of neurons.

41. A plasmid consisting in the deposit made at C.N.C.M. under the Accession Number I-2463.

42. A plasmid consisting in the deposit made at C.N.C.M. under the Accession Number I-2464.

43. A plasmid consisting in the deposit made at C.N.C.M. under the Accession Number I-2465.

44. A plasmid consisting in the deposit made at C.N.C.M. under the Accession Number I-2466.

45. A polynucleotide containing the sequence SEQ ID NO:6 add SEQ ID NO:6 from pages 15-16

46. The polynucleotide of claim 24, wherein said polynucleotide further comprises an heterologous amino acid sequence coding for an heterologous polypeptide under the control of NAP1L2 promoter.

47. A vector containing the polynucleotide of claim 46.

48. A neural cell containing the polynucleotide of claim 46.

49. A process for targeted expression of a polypeptide in a neural cell wherein said neural cell is a cell according to claim 48.

50. The polynucleotide of claim 32, wherein said polynucleotide further comprises an heterologous amino acid sequence coding for an heterologous polypeptide under the control of Nap1l2 promoter

51. A vector containing the polynucleotide of claim 50.

52. A neural cell containing the polynucleotide of claim 50.

53. A process for the targeted expression of a polypeptide in a neural cell wherein said neural cell is a cell according to claim 52.

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